Microbial catalysis of the oxygen reduction reaction for microbial fuel cells: a review.

Benjamin Erable, Damien Féron, Alain Bergel

Microbial Catalysis of the Oxygen Reduction Reaction for Microbial Fuel Cells: A Review

Benjamin Erable,[a] Damien Féron,[b, c] and Alain Bergel*[a]

The slow kinetics of the electrochemical oxygen reduction reaction (ORR) is a crucial bottleneck in the development of microbial fuel cells (MFCs). This article firstly gives an overview of the particular constraints imposed on ORR by MFC operating conditions: neutral pH, slow oxygen mass transfer, sensitivity to reactive oxygen species, fouling and biofouling. A review of the literature is then proposed to assess how microbial catalysis could afford suitable solutions. Actually, microbial catalysis of ORR occurs spontaneously on the surface of metallic materials and is an effective motor of microbial corrosion. In this framework, several mechanisms have been proposed, which are reviewed in the second part of the article. The last part describes the efforts made in the domain of MFCs to determine the microbial ecology of electroactive biofilms and define efficient protocols for the formation of microbial oxygen-reducing cathodes. Although no clear mechanism has been established yet, several promising solutions have been recently proposed.

1. Introduction

For approximately one century,[1] microbial fuel cells (MFCs) have been believed to be a promising technology for the production of electrical energy directly by the oxidation of organic matter. Since the discovery, early in the 21st century, of the capacity of microbial cells[2] and microbial biofilms[3] to catalyse electrochemical reactions, our understanding of microbial electrocatalytic mechanisms on anodes has advanced fast.[4] In comparison, few studies have been devoted to the development of MFC cathodes, even though they constitute a crucial bottleneck. Using oxygen as final electron acceptor would clearly be the most convenient solution to develop MFCs with wide applicability, but the kinetics of the oxygen reduction reaction (ORR) is slow. Different final electron acceptors, such as hexacynoferrate(IV) or permanganate, can give faster reduction kinetics than oxygen, but they would not afford sustainable options.[5]

The high value of the standard equilibrium potential of oxygen reduction,

$$O_2 + 4\,H^+ + 4\,e^- \rightarrow 2\,H_2O \qquad (1)$$

$E_0 = 1.229$ V measured versus standard hydrogen electrode (SHE) under standard conditions, makes it a ubiquitous final electron acceptor for a very large number of redox processes. Fortunately, the kinetics of ORR is slow and catalysts are rare.[6] If ORR kinetics were fast on materials readily available over the earth's surface, a wide variety of oxidation reactions would occur spontaneously. For example, metallic materials would corrode very rapidly and non-metallic materials might be highly sensitive to oxidative deterioration. The reactive oxygen species resulting from fast oxygen reduction would exert incredibly high oxidative stresses on living organisms, resulting in accelerated ageing and death. The rarity of efficient ORR catalysts on the surface of the earth can be seen as a necessary condition for the protection of living creatures in general and human beings in particular. These few general considerations give an idea of the difficulty of discovering efficient ORR catalysts.

ORR catalysts used in MFCs can be organised into three groups: chemical, enzymatic and microbial. Chemical catalysts have been directly derived from the work on conventional low-temperature fuel cells, notably proton exchange membrane fuel cells (PEMFCs). Platinum offers the highest catalytic performance, but has not allowed PEMFCs to become economically efficient yet. The limited availability of platinum, and therefore its cost, and the strong environmental impact linked to its production are serious drawbacks. Moreover, platinum is inhibited by numerous pollutants and consequently requires very pure fuels. Earlier studies of other chemical compounds for PEMFCs have met little success.

Living organisms have developed efficient oxidoreductases to achieve and control ORR. By taking advantage of these enzymes, mainly laccases and bilirubin oxidases,[7] it has been possible to design biofuel cells allowing ORR at potential values close to the E_0' value. Associating a microbial anode with a laccase-catalysed cathode has given the highest voltage reported for a MFC that uses oxygen as electron acceptor.[8]

[a] *Dr. B. Erable, Dr. A. Bergel*
Laboratoire de Génie Chimique, CNRS
Université de Toulouse
4 allée Emile Monso, BP84234, 31432 Toulouse (France)
E-mail: alain.bergel@ensiacet.fr

[b] *Dr. D. Féron*
CEA, DEN, DANS, DPC, SCCME
Centre de Saclay
bâtiment 458 - PC 50, 91191 Gif-sur-Yvette (France)

[c] *Dr. D. Féron*
LAMBE, Université d'Evry-Val-d'Essonne
boulevard François Mitterrand, 91025 Evry, Cedex (France)

In this respect, enzymes are likely the best catalytic option. Nevertheless, enzymes are very sensitive to any kind of inhibition, they often require sophisticated chemical operations to be immobilised on electrode surfaces and their lifetimes generally do not exceed a few days in operating conditions. From the current state of the art, it can be thought that enzyme catalysis is more suitable for disposable devices that should be able to deliver high power density immediately and for a short time, whereas microbial catalysis seems more suited to long-term production where a relatively long starting phase is acceptable.

A multi-criteria comparison of the different options of ORR catalysis has been proposed recently.[9] From a general point of view, microbial catalysis may offer very promising advantages[10] because its characteristics and constraints are similar to those of microbial anodes. Moreover, microbial catalysis of ORR is known to occur spontaneously on the surface of metallic materials that are exposed to natural environments, notably seawater. This phenomenon has been identified as the main motor of aerobic corrosion, which is called microbially influenced corrosion (MIC) in this case. The knowledge gained in the domain of MIC may offer a helpful basis for progressing in cathode design for MFCs.

The purpose of this article is to review the advances that have been made in understanding microbial catalysis of ORR in both MIC and MFC domains. Reviews on MIC generally devote a large part to the characterisation of material surfaces and passive layers,[11–13] whereas reviews on MFCs generally broaden their scope to abiotic oxygen-reducing cathodes[9,10] or to anaerobic microbial cathodes for synthesis and bioremediation applications.[14–17] The present article only focuses on ORR microbial catalysis while trying to build bridges between microbial corrosion and MFC thematic areas.

2. Basics on Oxygen Reduction Reaction in MFC Conditions

The electrochemical reduction of oxygen in an aqueous electrolyte can proceed by two overall pathways as represented in Table 1. Detailed mechanisms are more complex and they can involve many elementary steps with adsorbed species and radicals, which depend strongly on the electrode material.[6] The 4-electron pathway appears to be predominant on noble-metal electrocatalysts, whereas the peroxide pathway is predominant on graphite, gold, oxide-covered metals and most carbon materials.

ORR has been investigated in depth with a view to developing chemical fuel cells, mainly PEMFCs for low temperatures, but MFCs involve different constraints and requirements[5] that are listed below.

2.1. Neutral pH value

The abiotic cathodes developed for chemical fuel cells offer only limited performance in MFCs. These cathodes have been optimised to work in contact with a proton exchange membrane at extreme acidic pH values. A quick glance at the reaction equations shows that oxygen reduction is favoured by acid conditions, to provide the reaction with protons or to extract the hydroxyl ions produced. However, microbial development most often requires solutions at pH values ranging from 6.0 to 9.0. The common MFC operating conditions are consequently detrimental to ORR thermodynamics and kinetics.

Table 1. ORR overall pathways and standard potentials (from Ref. [6]).

Condition	Equations	Equation number	E_0 (vs. SHE) [V]
acid solution	*4-electron pathway*		
	$O_2 + 4H^+ + 4e^- \rightleftarrows 2H_2O$	1	1.229
	Peroxide pathway		
	$O_2 + 2H^+ + 2e^- \rightleftarrows H_2O_2$	2	0.67
	followed by the reduction of peroxide		
	$H_2O_2 + 2H^+ + 2e^- \rightleftarrows 2H_2O$	3	1.77
	or peroxide disproportionation		
	$2H_2O_2 \rightleftarrows 2H_2O + O_2$	4	
alkaline solution	*4-electron pathway*		
	$O_2 + 2H_2O + 4e^- \rightleftarrows 4OH^-$	5	0.401
	Peroxide pathway		−0.065
	$O_2 + H_2O + 2e^- \rightleftarrows HO_2^- + OH^-$	6	
	followed by the reduction of peroxide		
	$HO_2^- + H_2O + 2e^- \rightleftarrows 3OH^-$	7	0.867
	or peroxide disproportionation		
	$2HO_2^- \rightleftarrows 2OH^- + O_2$	8	

2.2. Slow oxygen transfer

Oxygen solubility is low in aqueous solutions. It depends on the temperature and salinity of the solution, being higher at low salinity and low temperature, but it should be borne in mind that oxygen solubility is around 1 mM in pure water, implying that a solution in contact with air contains only around 0.24 mM dissolved oxygen. Diffusion coefficients are within the common range of values for dissolved gases in aqueous solution, that is, $1–3\ 10^{-9}\ m^2\,s^{-1}$.[6] Mass transfer of oxygen to the electrode surface is consequently a slow step in MFCs, which must be considered with considerable care to improve cathode design. Air-breathing cathodes with a side in direct contact with air, a design coming from chemical fuel cell development, offer a relevant solution. Nevertheless, in MFCs the second face of air-breathing cathodes is in contact with the solution. The air-cathodes may be subject to problems of water "crossover", which provokes the decrease of catalytic performance.

2.3. Sensitivity of the microbial catalyst to reactive oxygen species

The peroxide pathway leads to hydrogen peroxide, with the possible production of other intermediate reactive oxygen species, such as the superoxide radical ion ($O_2^{\bullet -}$), which results from the mono-electronic reduction of oxygen. The hydroxyl radical ($OH^{\bullet}$) can also appear, particularly in the presence of iron ions, through the Haber–Weiss reaction

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\bullet} + OH^-$$

and the system can then cycle in the Fenton process. The reactive oxygen species can damage almost all components of living cells, including proteins, lipids and DNA. The overproduction of reactive oxygen species at the cathode can have immediate detrimental effects by killing the microbial catalysts of both cathode and anode. It should be noted that the reactive oxygen species are known to be responsible for similar long-term problems in PEMFCs, for example, deterioration of the proton exchange membrane.

2.4. Fouling due to alkalinisation of the interface and biofouling

The consumption of protons or the production of hydroxyl ions at the cathode surface results in alkalinisation of the interface. This is not a problem for PEMFCs, which use only hydrogen and oxygen. In contrast, MFC solutions contain many compounds and ions that precipitate when the pH value is decreased. Alkalinisation of the cathode interface can thus lead to severe fouling of the cathode surface by precipitation of hydroxides. The value of the interfacial pH depends on the balance between the rates of proton consumption and proton transport to the surface. Mass transport of protons must consequently be increased as much as possible in the vicinity of the cathode surface by stirring or buffering the solution. Nevertheless, it can be predicted that the decrease of the interfacial pH on the cathode surface will dramatically restrict MFC suitability for open environments that contain high ionic concentrations. For example, the presence of calcium ions in seawater results in cathode fouling by precipitation of calcium hydroxides when the current density increases. High current densities could be reached only in media that do not contain species that precipitate at the interfacial pH value.

Biofouling should also be noted as a possible cause of decreased performance in open environments. Biofouling can easily be induced by influents that contain organic matter, even at low concentration, causing thickening of the biofilm and a dramatic decrease in the mass transfer rates of ionic species.

3. History: From MIC to MFC

LaQue[18] and then Nikita and Ulanovskii[19] pointed out that the open circuit potential of stainless steels increased with exposure time in natural seawater. This phenomenon, called "ennoblement of free (corrosion) potential" in the field of corrosion, has considerable economic importance because it shifts the material from passivity to an electrochemical state at which corrosion can occur. Ennoblement of free potential is commonly of the order of $+300$ mV and can reach $+500$ mV.[20] It has been observed in seawaters from tropical[21] to Antarctic conditions;[22] in a large diversity of water bodies[13] such as rivers,[23,24] estuaries[25] and wastewater plants;[26] and with different metallic materials.[27] In 1976, Mollica and Trevis[28] correlated the free-potential ennoblement to the formation of a microbial biofilm that enhanced the cathodic branch of the exchange currents. From this date, it was widely agreed upon that biofilms formed in aerated waters spontaneously catalysed ORR on metallic surfaces. The biofilm effect[29] or the role of ORR catalysis[23] have sometimes been denied in a few studies, but the great majority of studies have postulated microbial catalysis of ORR.[27,30]

Corrosion studies have commonly been carried out in open-circuit conditions with the objective of investigating the effect of ORR catalysis on the free potential. In this type of experiments only the exchange currents are involved, which have very low values. To the best of our knowledge, the first oxygen-reducing microbial cathodes designed with the aim of increasing current densities were reported in parallel by two different groups in 1997. Mollica's group[31] showed that stainless-steel electrodes polarised at -0.2 V versus saturated calomel electrode (SCE) for a few days in seawater provided current densities of around 0.2 A m^{-2}. The same procedure repeated at eleven different European sea sites, with polarisations of 0.0 V versus SCE, gave current densities from 0.01 to 0.1 A m^{-2}, whereas less than 10^{-5} A m^{-2} was measured in the absence of a biofilm.[32] In parallel, a similar seawater oxygen-reducing biofilm was developed on a graphite brush and coupled to a magnesium alloy anode in the design of a submarine battery.[33]

The seawater microbial cathode was then adapted to a fuel cell, giving current densities of up to 1.89 A m^{-2} with aerated seawater.[34] As was the case for corrosion, microbial cathodes for fuel cells have now been formed with various inocula (see Table 2). The knowledge gained in the corrosion domain allowed the first pure-strain oxygen-reducing cathode to be designed.[58] Pure cultures still remain poorly explored, although the highest current density has been reported with a pure strain of *Acidithiobacillus ferrooxidans* grown on a graphite electrode, which gave rise to 5.0 A m^{-2} at 0.0 V versus SCE working at pH 2.0 under a pure oxygen atmosphere.[54] It appears that a majority of studies devoted to ORR microbial catalysis has now shifted from the domain of corrosion to the MFC area.

4. Mechanisms of ORR Microbial Catalysis Identified in MIC

4.1. Direct catalysis by extracellular enzymes released by microbial cells

Pioneering research in aerobic MIC was aimed at identifying the components of the seawater biofilms that could be linked to microbial ORR catalysis.[59] Potential ennoblement was found to be more closely correlated to the quantity of carbohydrates and proteins contained in the biofilm than to the number of settled bacteria. Moreover, the addition of sodium azide, a strong inhibitor of the enzymes of the respiratory chain, to natural seawater decreased the free potential from $+350$ to $+100$ mV versus SCE. It has been concluded that ORR microbial catalysis was due to extracellular proteins, such as enzymes, which were adsorbed on the material surface (Scheme 1, reaction 1). Superoxide dismutases, catalases and peroxidases, which respectively catalyse

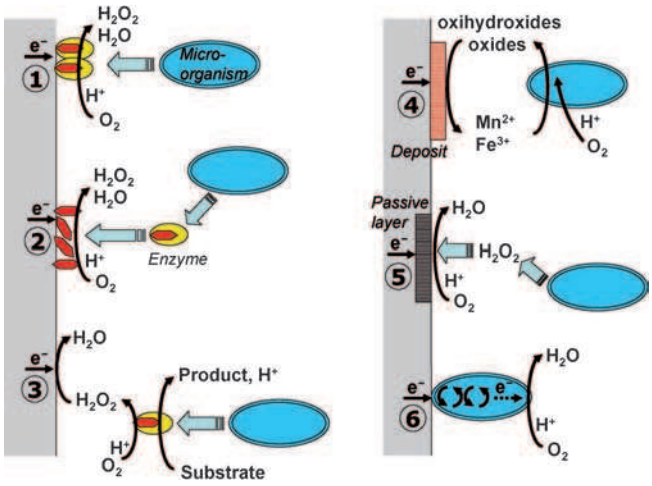
Table 2. Electrode materials, inoculum sources, operating conditions and performances of oxygen-reducing microbial cathodes.

Ref.	Cathode	Inoculum source	Operating mode	Electrochemistry	Free potential [mV]	P [$W\,m^{-3}$]	J [$A\,m^{-2}$]	Time
Waste water								
[35]	carbon felt	sludge/sediment mix	continuous (6 $L\,h^{-1}$) M9 medium	MFC (100 Ω)	$>+400$[a]	3[b]	60[b]	7 months
[36]	plain granular graphite	anaerobic/anoxic sludge	continuous (1.3 $mL\,min^{-1}$) nutrient solution, nitrate 350 days	MFC (30 Ω)		6.5[b]	34.46[b]	40 days
[37]	glassy carbon	activated sludge	continuous synthetic wastewater	MFC (250 Ω)		60	159	10 months
[38]	carbon felt	aerobic sludge	batch-fed mode phosphate buffer solution	IP ($+0.242$ V[a])		0.11[b]	0.85	27 days
[39]	graphite fibre brush	aerobic activated sludge	batch-fed mode nutrient solution	MFC (100 Ω)	0.482[a]	68.4[b]	325[b]	233 h
[40]	graphite felt membrane electrode assemblies	aerobic sludge	batch-fed mode phosphate buffer 144 mL	MFC (51 Ω)		16.7[b]	4.0[b]	100 days
[41a]	plain granular graphite	anaerobic/anoxic sludge	continuous (13 $mL\,min^{-1}$) synthetic wastewater nitrate 350 days, oxygen 50 days	MFC (30 Ω)		10.3[b]	44.2[b]	400 days
[41b]	graphite granules	aerobic sludge	continuous (0.2 $mL\,min^{-1}$) anodic effluent	MFC		2.55[b]	20[b]	180 days
[42]	granular graphite 6 mm diameter	acetate-fed MFC (200 days) origin: activated sludge	batch-fed mode synthetic medium recirculation loop (8 $L\,h^{-1}$)	IP (in MFC, -0.3 V[c])			0.040[d]	<1 day
[43]	rough graphite plates	nitrifying biomass (WTP)	recirculation 12 $mL\,h^{-1}$	IP (in MFC, $+0.150$ V[c])			313	20 days
[44]	semicoke Carbon granules activated carbon granules graphite carbon felt	consortiums previously enriched in bio-cathode MFC origin: anaerobic/aerobic sludge	batch-fed mode 3 days (synthetic medium) mixing by recirculation (20 $mL\,min^{-1}$)	MFC (1000 Ω)		20.1[b] 24.3[b] 14.1[b] 17.1[b]		3 months
Seawater/freshwater								
[34]	stainless steel UNS S31254	seawater	on site open seawater	IP (-0.3 V[a])			460	12 days
[45a]	stainless steel UNS S31254	seawater	on site open seawater	MFC (33 Ω)		0.023	0.140	3 months
[46b]	stainless steel UNS S31254	seawater	continuous (6 $L\,h^{-1}$) air sparging	MFC (25 Ω)			0.140	60 days
[47]	graphite felt	mixture of environmental samples from river-rusted metal	continuous (4 $L\,h^{-1}$) modified M9 medium				0.996	48 days
[48]	carbon felt stainless steel 316 L	aliquots of water from previous biocathodes origin: sediments, soil, river water, sludge, MOB	batch-fed mode air sparging	OCP - MFC (500 Ω)	548 545	0.280 0.020	0.940 0.360	60 days
[49]	stainless steel 254SMO	seawater/wild aerobic marine biofilm *Acinetobacter Johsonii* *Winogradskyella poriferorum*	batch–continuous (60 $mL\,h^{-1}$) seawater	IP (-0.2 V[c])			0.020–0.600 5 10	10–40 days 10 days 10 days
[45b]	stainless steel super austenitic	seawater	batch-fed mode 2 L–200 L–2000 L	OCP–IP	$<+350$[c]		0.600	16 days
[50]	graphite plates	sediment/water	batch-fed mode	SMFC–OCP	506[a,f] 485[a,g]		0.470[a,e,f] 0.3a[c,e,g]	45 days
[51]	carbon felt	freshwater (500 mL)/sediment (700 g)	batch-fed mode	MFC (1000 Ω)	<450[a]	0.034		40 days

Table 2. (Continued)

Ref.	Cathode	Inoculum source	Operating mode	Electrochemistry	Free potential [mV]	*P* [W m^{-3}]	*J* [A m^{-2}]	Time
Soil								
[52]	graphite mats	paddy soil/rice roots	batch-fed mode	MFC (1000 Ω)	0.200[h] 0.750[h]			30 days
[53]	graphite fibre brush and carbon granules	topsoil	batch-fed mode	MFC (500 Ω)	500[a]	100[b]	>400[b]	400 h
Pure culture								
[47]	graphite felt	mixture of environmental samples from river-rusted metal	batch-fed mode modified M9 medium				0.690 300–640	
[54]	graphite felt	*Acidithiobacillus ferrooxidans*	batch-fed mode modified Mackintosh medium pH 2.0	IP (0 V[a])			5	28 days
[55a]	glassy carbon	*Pseudomonas aeruginosa*	batch-fed mode phosphate buffer 0.1 M pH 7.0	CV			2	1 hour
[55b]	glassy carbon	*Pseudomonas aeruginosa*	batch-fed mode	CV			1.671	1 hour
		Pseudomonas fluorescens	phosphate buffer 0.1 M pH 7.0				1.428	1 hour
		Brevundimonas diminuta					2.028	1 hour
		Burkholderia capacia					2.442	1 hour
		Branhamella catarrhatis					1.914	1 hour
		Enterobacter cloacae					1.471	6 h
		Escherichia coli					1.457	3 h
		Shigella flexneri					1.642	3 h
		Acinetobacter sp.					1.442	1 hour
		Kingella kingae					1.371	3 h
		Kingella denitrificans					1.714	1 hour
		Micrococcus luteus					1.928	1 hour
		Bacillus subtilis					1.657	1 hour
		Staphylococcus carnosus					1.357	1 hour
[49]	stainless steel 254SMO	*Acinetobacter Johsonii*	batch-fed mode	IP (−0.2 V[c])			0.005	10 days
		Winogradskyella poriferorum	seawater				0.010	10 days
[56]	carbon paper	*Acinetobacter calcoaceticus* NBRC 12552 *Shewanella putrefaciens* NBRC 3908	batch-fed mode nutrient medium	IP (in MFC, −0.200 V[a])			4×1^{-5} 10^{-5}	<1 day
[57]	activated granular carbon 2.5-4 mm diameter	ferro/manganese oxidising bacteria	continuous (5 mL min^{-1}) batch-fed mode nutrient phosphate buffer-based solution	MFC (100 Ω)		32[b] 28[b]	79[b]	350 h

[a] Measured versus SHE. [b] Per m^3. [c] Measured versus Ag/AgCl. [d] Current in A. [e] 250 mV. [f] Seawater. [g] River. [h] Open circuit voltage.



Scheme 1. Different mechanisms postulated to explain the microbial catalysis of oxygen reduction by biofilms. 1) Direct catalysis by adsorbed extracellular proteins. 2) Direct catalysis by adsorbed prosthetic groups (porphyrins) or metal-exopolymer compounds. 3) Indirect catalysis by enzymes that reduce oxygen to hydrogen peroxide and organic acid. 4) Indirect catalysis mediated by manganese or iron oxides produced by ferro/manganese bacteria. The bacteria reduce oxygen and oxidise iron or manganese ions to oxihydroxides or oxides, which are reduced back to ions on the electrode surface. 5) Production of hydrogen peroxide by the biofilm improves the oxygen-reducing catalytic properties of the oxide layers of stainless steels. 6) Direct electron transfer from the electrode to the bacterial cell; this mechanism has been postulated by analogy with anaerobic cathodes.[16]

-disproportionation of the superoxide radical ion

$$2\,O_2^{\bullet -} + 2\,H^+ \rightarrow O_2 + H_2O_2 \qquad (9)$$

-disproportionation of hydrogen peroxide

$$2\,H_2O_2 \rightarrow O_2 + 2\,H_2O \qquad (10)$$

-and oxidation of several substrates (noted X-H_2)

$$2\,X\text{-}H_2 + H_2O_2 \rightarrow 2\,X\text{-}H^{\bullet} + 2\,H_2O \qquad (11)$$

were guessed to be possible extracellular enzymes able to catalyse ORR. Bioelectrochemical studies have confirmed that catalase and horseradish peroxidase adsorbed on glassy-carbon and pyrolytic-graphite electrodes catalyse ORR by direct electron transfer.[56,60]

4.2. Direct catalysis by porphyrins and organometallic compounds entrapped in microbial biofilms

Porphyrins constitute the prosthetic group of catalase and several oxidases. Adsorbed iron porphyrin has been shown to catalyse ORR on glassy-carbon electrodes.[56,61,62] On stainless steel, micro-sized spots of iron porphyrin have induced local catalysis of ORR.[63] It can consequently be thought that the prosthetic group of oxidases, which would remain adsorbed on the electrode surface after enzyme degradation, can play the role of the ORR catalyst inside natural biofilms (Scheme 1, reaction 2). Similarly, organometallic complexes can be formed by complexation of metallic cations by the polysaccharides, which form an important part of the biofilm matrix.[11,64] Such organometallic complexes have been suggested as possible ORR catalysts.[30a,65,66] However, it has been observed that free-potential ennoblement is decreased by enzymatic inhibitors such as sodium azide,[59] which suggests that functional enzymes are involved in ORR and metallic complexes can only play a secondary role in natural biofilms.

4.3. Indirect catalysis mediated by hydrogen peroxide produced by the microorganisms

It has been pointed out that the presence of hydrogen peroxide at the biofilm/electrode interface is a key parameter for free-potential ennoblement.[67] It has been postulated that three conditions were required to reproduce potential ennoblement in artificial seawater: The solution must be acidic (pH value around 2.9), partially deoxygenated and must contain hydrogen peroxide (2.4 mM). Hydrogen peroxide can be produced by marine bacteria,[68] and hydrogen peroxide has often been detected in marine and fresh water biofilms.[69] Concentrations of 0.14–0.73 mM[67] and up to 6 mM[70] have been measured in natural marine biofilms. In parallel, an experimental model has been designed using glucose (10 mg L^{-1}) and glucose oxidase (GOx, Scheme 1, reaction 3). The GOx-catalysed reduction of oxygen led to hydrogen peroxide:

$$C_6H_{12}O_6 + O_2 + H_2O \rightarrow C_6H_{12}O_7 + H_2O_2 \qquad (12)$$

which induced free-potential ennoblement of stainless steels. The model assumes that oxidases are present in wild biofilms and produce hydrogen peroxide by oxidising organic compounds. The same experimental model has been implemented with glucose (1 mg L^{-1}) and glucose oxidase in sterilised or synthetic seawater.[71] It has thus been confirmed that acidification, which was ensured by the gluconic acid ($C_6H_{12}O_7$) produced, contributes to the ennoblement effect. A role of the (semi-)conductive properties of the oxide layers has also been evidenced.[71c] The glucose/glucose oxidase model has now been widely used to reproduce ORR microbial catalysis in aerobic MIC studies.[71d]

4.4. Indirect microbial catalysis mediated by manganese or iron oxides

In environments containing manganese or iron ions, ferro/manganese oxidising bacteria can oxidise these ions to oxides, which are reduced back to ions on metallic surfaces (Scheme 1, reaction 4). Such a cycling of manganese ions by manganese oxidising bacteria (MOBs) results in an electron transfer chain from the material to oxygen, which is the final electron acceptor of MOBs. The system has been widely investigated as a possible mechanism of aerobic MIC.[23,72] In more details, MOBs use oxygen to reduce manganese ions to manganese oxohydroxide (MnOOH), which deposits on the electrode surface and then leads to manganese dioxide (MnO_2). On the electrode surface, manganese dioxide is electrochemically

reduced back into Mn^{2+} ions with MnOOH as an intermediate species. The electrochemistry of manganese is complex, but it has been suggested that the reduction of manganese oxide into oxyhydroxide,

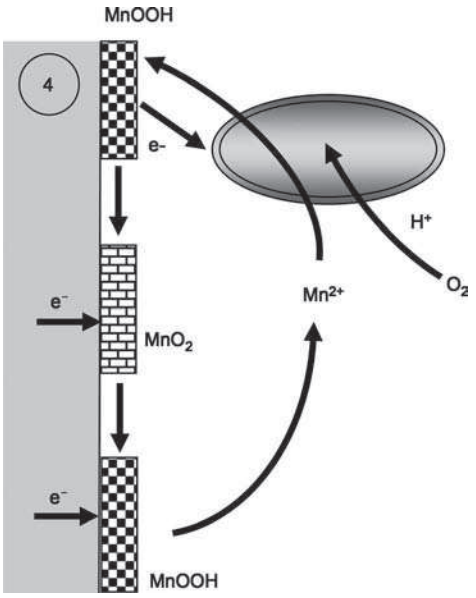
$$MnO_2 + H_2O + e^- \rightleftharpoons MnOOH + OH^- \quad (13)$$

with a standard equilibrium potential of 0.335 V versus SCE at pH 8.0, should be the key reaction that controls the free potential of the samples. This equation involves only solid deposited species, which can explain why the free potentials of metallic coupons have similar values in all field experiments. For example, the reduction of manganese oxide into manganese ions

$$MnO_2 + 2\,H_2O + 2\,e^- \rightleftharpoons Mn^{2+} + 4\,OH^- \quad (14)$$

has been ruled out because its standard equilibrium potential depends on the manganese ion concentration: 0.235 V versus SCE at pH 8.0 with 0.3 μM Mn^{2+}[23] or 0.310 V versus SCE at pH 7.5 with 0.1 mg L^{-1} Mn^{2+}.[73] This reaction would consequently result in ennoblement values fluctuating as a function of the concentration of the Mn^{2+} ions in different natural waters.

The model was confirmed by lab experiments with a MOB-pure culture.[73] Surface analyses demonstrated that the potential was controlled by the ratio between the amount of MnOOH and MnO_2 deposited on the material surface (Scheme 2). A large experimental campaign demonstrated that the concentration of manganese ions directly influenced the rate of ennoblement, whereas the concentration of dissolved oxygen had no significant effect.[74]



Scheme 2. Detailed pathway of the indirect microbial catalysis mediated by manganese compounds. The reaction between the deposited manganese hydroxides and manganese oxide controls the free potential of the electrode.[73]

4.5. Modification of the catalytic properties of iron oxides by seawater biofilms

The composition of the passive layer of stainless steels depends on the nature of the steel and is significantly affected by the medium in which the material is immerged. Studies of different stainless steels immersed in natural and artificial seawaters[75] have pointed out a thickening and a stratification of the passive layer. Hydrogen peroxide concentrations of 3–8 mM were measured in a two-month-old biofilm, and it has been postulated that hydrogen peroxide partially reduces the surface iron oxides, transforming trivalent iron atoms into divalent ones, which are known to be better ORR catalysts. According to this model, hydrogen peroxide produced by mature biofilms has an indirect effect by improving the catalytic properties of the iron oxides (Scheme 1, reaction 5).

4.6. Local acidification inside mature biofilms

Authors do not agree on whether or not marine biofilms can cause a local pH-value decrease on the material surface. Some authors have observed drastic acidification beneath some parts of the biofilm,[76] whereas others have claimed that the buffer power of seawater cannot allow the pH value to decrease significantly even in mature biofilms.[77] It has even been observed that ennoblement could be eliminated at low pH values.[27] The possible (or not) local acidification assumption remains a debated topic.

4.7. Influence of light

The possible influence of light on free-potential ennoblement remains uncertain. Some authors have denied any significant role of light,[78] others have shown that, after 4 h exposure to natural sunlight, seawater no longer causes free-potential ennoblement, whereas the amount of hydrogen peroxide produced is approximately doubled. Experiments performed with a Hg–Xe light source have suggested that light may also act on the nature of the oxides of the passive layer of steels.[79] It may be guessed that some possible effects of light that are not generally controlled during field experiments might be the cause of unexplained discrepancies among the results from different teams.

5. Microbial Catalysis of ORR in MFC

5.1. Ecology of oxygen-reducing microbial cathodes

Since 1997[31] and with renewed interest[34] due to the emergence of MFCs, the exploitation of microbial biofilms has been proposed as an effective solution for catalysing ORR around neutral pH at room temperature. The first studies have implemented aerobic biofilms formed from seawater on stainless steel electrodes. Now, microbial oxygen-reducing cathodes have been designed from many other sources of inoculum that can be divided into three groups: (i) wastewater and aerobic sludge,[36,37,41,80] (ii) seawater and freshwater[47–49] and (iii)

soil.[53] These environments are known to offer broad bacterial diversity.[81] In comparison to the work performed in the MIC area, a large amount of work devoted to MFCs has been turned towards the identification and isolation of bacterial species and systematic molecular characterisation of bacterial communities. The large ecological diversity of the ORR-catalysing biofilms is highlighted in Table 3.

5.1.1. Enrichment and isolation of pure strains

A few studies have attempted to isolate pure cultures from wild multi-species biofilms.[47,49] Bacterial isolations were always preceded by a phase of enrichment that promotes the growth of a given type of microorganism selected according to the physicochemical and nutritional conditions of the medium. Techniques that allow access to individual strains introduce a bias inherent in culture-dependent methods in two ways. Depending on the medium and the culture conditions, the growth of some species/genera/families is favoured. Moreover, only a very small proportion of the microorganisms contained in wild environments is cultivable. It has been assessed that only 0.001–0.1 % of the wild bacteria contained in seawater can be cultivated using conventional microbiology tech-

Table 3. Bacterial diversity highlighted from ORR catalysing biofilms.

Ref.	Cathode material	Electrochemistry	Film age	Inoculum source	Population analysis of biofilms ([%])
wastewater					
[36]	plain granular graphite	MFC (30 Ω)	40 days	anaerobic/anoxic sludge	proteobacteria (50) bacteroidetes (21.6), alphaproteobacteria (9.5), chlorobi (8.1), deltaproteobacteria (4.1), actinobacteria (4.1 %), gammaproteobacteria (2.6 %)
[37]	untreated glassy carbon	MFC (250 Ω)	10 months	activated sludge	firmicutes, alphaproteobateria, betaproteobacteria, gammaproteobacteria, bacteroidetes
[41a]	plain granular graphite	MFC (30 Ω)	400 days	anaerobic/anoxic sludge	proteobacteria, bacteroidetes, actinobacteria, planctomycetes, firmicutes, uncultured bacteria
[61b]	graphite granules	MFC	180 days	aerobic sludge	> predominant: deltaproteobacteria
seawater/freshwater					
[47]	graphite felt		48 days	mixture of environmental samples from river-rusted metal	bacteroidetes, alphaproteobacteria, gammaproteobacteria
[48]	carbon felt stainless steel 316L	OCP–MFC (500 Ω)	60 days	aliquots of water from previous biocathodes origin: sediments, soil, river water, sludge, MOB	pseudomonas ralsronia gammaproteobacteria cyanobacteria > different EA population between carbon and SS
[49]	stainless steel 254SMO	IP (−200 mV vs. Ag/AgCl)	10–40 days	seawater/wild aerobic marine biofilm	alphaproteobacteria gammaproteobacteria firmicutes acitobacteria flavobacteriaceae > main Gram negative bacteria
[82]	stainless steel	OCP	35 days	river water	actinobacteria firmicutes bacteroidetes alphaproteobacteria betaproteobacteria gammaproteobacteria
soil					
[53]	graphite fibre brush graphite granules	MFC (500 Ω)	400 h	topsoil	*Nitrobacter* sp., *Achromobacter* sp., *Acinetobacter* sp., bacteroidetes > chemoautotrophic bacteria

niques.[83] The ratio is around 0.25% for freshwater and sediments and up to 15% to activated sludge.

Rabaey et al.[47] first isolated autotrophic strains from an ORR-catalysing biofilm in 2008. A few isolates (*Sphingobacterium* sp. and *Acinetobacter* sp.) showed electroactive properties, but they led to current densities lower than those provided by the wild parental biofilm. A similar behaviour was observed with marine electroactive biofilms.[49,84] Among 30 heterotrophic bacterial strains isolated from wild ORR-catalysing marine biofilms, only two (*Winogradskyella johsonii* and *Acinetobacter poriferorum*) proved able to catalyse ORR, giving current densities of only a few percent of the current obtained with the parental wild biofilm. The hypothesis of a synergistic effect between the different microbial species making up the wild biofilm can be put forward. Other possible explanations can be pH change, surface modification or underdeveloped biofilm growth under pure culture conditions.[15]

5.1.2. Molecular phylogeny of wild complex biofilms

Culture-independent techniques are based on the analysis of nucleic acids (genomic DNA or 16S ribosomal RNA). The tools of molecular biology such as cloning and sequencing of 16S rRNA,[36,41,47] fluorescence in situ hybridization (FISH)[53] or denaturing gradient gel electrophoresis (DGGE)[37,49,53] have been largely implemented to analyse the microbial communities of ORR-catalysing biofilms (Table 3).

Analyses of the community structure and composition by molecular ecology techniques have revealed a high phylogenetic diversity of the ORR-catalysing biofilms. Phylogenetic groups detailed from oxygen-reducing microbial cathodes are mainly *alpha-*, *delta-* and *gamma-Proteabacteria*. Less known groups, such as *Bacteroidetes* and *Firmicutes*, have also been reported to be associated to these predominant groups (Table 3). These two groups have principally been highlighted in electroactive biofilms obtained from activated or anoxic sludge inocula.[36,41,47] In addition, novel and unculturable bacteria appear to be enriched.

Studies carried out on seawater microbial cathodes have shown no difference between the microbial composition of the biofilms that were able to catalyse ORR and those that were not.[45] In addition, the microbial population of the biofilms and of the surrounding seawater had the same dominant members.[84] It is consequently difficult to draw any firm conclusion, except that it has not yet been possible to establish a correlation between the electroactivity of aerobic biofilms and their microbial composition.[45]

5.2. Implementation in MFCs and performance

5.2.1. Biofilm formation: Polarisation, open circuit, low-resistance-connected MFC

The first phase of biofilm formation plays an important role in the evolution and performance of the cathode in a MFC. The first strategy proposed for forming efficient microbial cathodes consisted of polarising the electrode at a potential value low enough to induce a cathodic behaviour.[31,20,32b] A current density of 1.89 $A\,m^{-2}$ was thus obtained with stainless steel cathodes in seawater maintained under air-bubbling.[34] These experiments used a three-electrode set-up with a potentiostat controlling the potential of the cathode versus a reference electrode.

Biofilms were then formed under "natural" conditions or, in other words, on electrodes left at open circuit.[46,48,50] In this case, the open circuit potential (OCP) value corresponds to the so-called free potential in the domain of corrosion. Similarly, it was also possible to connect the cathode and the anode of a MFC through a high resistance ($R > 1000\ \Omega$).[51,52,85] The electrode potential remained close to the OCP value, but it was slightly influenced by the potential of the anode. As the microbial anode forms concomitantly with the cathode, the potential of the cathode may vary erratically. In both cases (open circuit or high resistance) the availability of electrons from the cathode is very low and the growth of electroactive species that would use the electrode as electron source is not specifically favoured.

With a view to increasing the availability of electrons at the cathode and thus promoting development of electroactive species, a strategy consists in connecting the anode and cathode through a low resistance ($R < 1000\ \Omega$).[35,37,39–41,47] The cathode potential is thus attracted to that of the anode, generally in the range of -300 to -400 mV versus SCE. Nevertheless, the potential is not controlled and changes depend on the rates of biofilm development on the anode and the cathode. It has been suggested that the control of the electrode potential could play a role in microbial physiology, including changes in cell surface properties, an increase in enzymatic activity and a shortening of the generation time of bacteria. This certainly explains why potential control is widely used in the most recent studies.[38,42,43,45,49,54,56] Nevertheless, it has recently been reported[45] that marine biofilms formed on stainless steel electrodes, either under polarisation or at open circuit, supported a similar ORR performance when they were finally polarised, providing current densities of about 0.6 $A\,m^{-2}$ for several weeks. The best strategy for forming efficient oxygen-reducing microbial anodes remains open to debate.

5.2.2. Electrode material

Carbon and graphite materials are used in most studies on oxygen microbial cathodes. A few studies have used flat, smooth electrode structures such as graphite plates,[43,50] glassy carbon[37] or carbon paper,[56] but the majority has implemented three-dimensional (3D) structures, such as the widely-used carbon felts,[35,38,40,47,48,51,54] graphite granules[36,41,57,85] and graphite fibre brush.[33,39] Zhang et al.[53] even combined graphite fibre and granules to create a new generation of graphite-fibre-based cathode, in which the brush played the dual role of biofilm support and current collector for granules. Wei et al.[85] compared several carbon-based supports using a mix of aerobic and anaerobic sludge as inoculum. The different carbon-based electrodes offered structures with increasing surface areas: plain graphite, carbon felt, carbon granules and ac-

tivated carbon granules. The higher the developed surface area of the carbon support was, the greater was the current density obtained. However, a linear relationship between the surface area available for biofilm growth and the current density was not obtained.

It is difficult to compare performance when it comes to 3D structures. The surface areas really active are clearly increased and consequently the current densities need to be expressed in $A\,m^{-3}$, rather than $A\,m^{-2}$. Moreover, when using volumetric current densities, care should be taken to express the current with respect to the electrode volume and not versus the total reactor volume. The first is relevant for 3D structures, whereas the second would not make sense and would only result in wrong comparisons.

Beyond carbon or graphite electrodes, stainless steel has also often been used as a support electrode.[34,45,46,48,49] There are very few studies comparing electrode materials in strictly identical experimental conditions. Carbon felt cathodes have been reported to give an approximately three times higher performance, with current densities of about $1\,A\,m^{-2}$, than stainless steel.[48] Nevertheless, it should be noted that the specific surface area of the carbon felt can be 10^3 times that of the stainless steel.[14]

5.2.3. Fed-batch versus continuous catholyte flow

For nutrient-poor environments such as seawater or freshwater, the availability of nutrients in the solution can drastically affect the electrode performance and stability. This consideration depends on the "electrode surface area/bulk volume" (*A*/*V*) ratio. A current density limited to $20\,mA\,m^{-2}$ was provided by a marine biofilm formed in aerated seawater. The current was stable for only six days due to nutrient depletion. In this case, the *A*/*V* ratio was rather high, that is, $5\times10^{-2}\,cm^{-1}$.[49] In contrast, the same experimental system implemented in continuous mode ($60\,mL\,h^{-1}$) reached $600\,mA\,m^{-2}$ with a stability of more than 40 days. Similarly, such nutrient limitations were observed with similar marine cathodes implemented with lower *A*/*V* ratios in the range of 1.2×10^{-5}[45] to $7.6\times10^{-3}\,cm^{-1}$.[48]

For richer media (anodic effluent, nutrient-supplemented solution, synthetic wastewater), the feeding mode does not significantly affect the performance of microbial cathodes. Many of the studies carried out with rich media have been performed by means of continuous feeding or at least by using a catholyte recirculation loop. Continuous feeding allowed a maximum concentration of oxygen to be ensured in the catholyte and the thickness of the biofilm to be controlled by hydrodynamic erosion.[43] Other studies have started in fed-batch mode for a few days before switching to continuous mode.[42,44] A study dealing with a Mn^{II}- and Fe^{II}-mediated system did not reveal any difference between fed-batch and continuous-feeding modes.[57]

6. Future for Microbially-Catalysed ORR: Targets and Outlook

6.1. About mechanisms

The MIC and MFC domains have developed complementary investigation approaches. The former have put their main efforts into looking for the component(s) and the mechanism(s) responsible for "free (corrosion)-potential ennoblement", whereas the latter have focused on the microbial composition of the biofilms and the isolation of bacterial strains. Nevertheless, no correlation has been evidenced yet between the microbial composition of aerobic biofilms and their capability to catalyse ORR.

No considerable advances have been made in the fundamental understanding of mechanisms in MFCs with respect to the knowledge gained in the MIC domain. The "mediation by manganese oxides" model discussed in MIC (section 4.4) has been confirmed and exploited to design specific cathodes.[35] Biomineralised manganese oxide deposited by a pure bacterial strain (*Leptothrix discophora*) has been observed on graphite cathodes, which provided currents two orders of magnitude higher than the clean electrode.[58] Cathodes elaborated by impregnating their surface with iron and manganese have also shown an increase in ORR catalysis, which was associated with the presence of ferro/manganese-oxidising bacteria in the biofilm.[57] In addition, studies carried out in pure cultures with collection strains[23] and isolates[62] have confirmed the probable involvement of catalase or porphyrinic compounds in the catalytic pathway (sections 4.1 and 4.2).

A huge number of isolates and collection strains have been tested by cyclic voltammetry. Among 32 bacterial isolates coming from electroactive phototrophic river biofilms, 25 have thus shown their ability to induce transient catalysis of ORR.[82] Several seawater isolates,[62] many collection strains[55,56] and even strains coming from anoxic biofilms[86] have shown similar transient catalysis. The very different phenotypic properties of these strains (gram stain, oxidase, catalase…) tend to confirm the involvement of a ubiquitous compound such as porphyrin. Actually, the voltammetry experiments involved adhered bacterial cells only, and no structured biofilm was formed on the electrode surface during these tests. This transient analytical technique detected a catalytic compound, but it did not prove the presence of a metabolic pathway that could support stationary oxygen reduction. The majority of these cells were not able to form ORR-catalysing biofilms. It must be concluded that the compound detected by voltammetry does not necessarily confer the ability to achieve stable oxygen reduction under constant polarisation. Voltammetry may consequently detect a secondary catalytic pathway, which bypasses the respiratory chain. This pathway may be due to excreted compounds, extracellular porphyrinic enzymes and their prosthetic groups, for example, which are produced to protect the cells against oxidative stress. In this case, other pathway(s) that require live bacteria should exist.[56,62] In the same way, Rosenbaum et al.[16] have suggested that the reduction of oxygen does not necessarily require enzymes, postulating that microbi-

al cofactors, such as heme molecules, can be involved. By analogy to anaerobic cathodes, it may be thought that direct electron transfer may occur from the electrode to the bacterial cell[16] (Scheme 1, reaction 6), but this pathway still remains to be demonstrated.

The nature of the electrode material and its surface state are likely to have a significant effect on the performance of microbial cathodes, mainly in the final electron transfer from the biofilm to the electrode surface. Studies investigating this topic remain rare. For example, by using a stainless steel electrode, the electronic state of the surface oxide layer has been highlighted as a significant parameter that affects the current provided by seawater microbial cathodes in both, laboratory tests[71c,46a] and a marine MFC pilot.[46b] A complex interface elaborated by electrodepositing carbon nanotubes and chitosan nanocomposite on a carbon electrode has been shown to enhance electron transfer between the electrode and the ORR-catalysing biofilm.[87] Increasing research efforts on this aspect would certainly afford significant advances.

Clearly, better understanding of the fundamental mechanisms remains a strong need. In this framework, identifying a pure strain, or a mixed culture if synergetic effects are essential, that could serve as experimental model would constitute a considerable progress.

6.2. Applications

The number and diversity of the constraints listed in Section 2 of this article may encourage pessimism. In contrast, the brief review of the studies devoted to oxygen-reducing microbial cathodes shows that clever and multiple tracks have been opened up to solve them.

6.2.1. pH value

The slow ORR kinetics at neutral pH might be solved in different ways. The acidophilic strain *Acidithiobacillus ferrooxidans*, which produces 5 $A\,m^{-2}$ at 0.0 V versus SCE at pH 2.0, is a very promising option.[54] It should be associated with an acidophilic anode-respiring bacterium at the anode or would require a particular MFC design with an acidic cathode chamber.[88]

In an MFC, oxidation of the fuel, which is usually acetate for laboratory cells,

$$CH_3COO^- + 4\,H_2O \rightarrow 2\,HCO_3^- + 9\,H^+ + 8\,e^- \qquad (15)$$

results in acidification of the anode chamber, whereas oxygen reduction causes alkalinisation of the cathode side. This is a general problem for MFCs, which is solved in laboratory conditions by using buffered solutions. It has been proposed that the pH value of the cathode should be controlled by sequentially feeding the cathode with the effluent of the acetate-fed anode. In this way, the protons produced at the anode side are directly introduced into the cathode compartment to enhance ORR. Such an experiment showed a four-fold increase of the current provided by the microbial cathode during nine months of operation.[89]

In this framework, the reversible anode concept is also of great interest. Cord-Ruwisch's group demonstrated that a microbial electrode formed from activated sludge and maintained at −0.3 V versus Ag/AgCl was able to repeatedly change from anode to cathode function when the supply alternated between acetate and dissolved oxygen.[80] The protons produced during the anode phase by acetate oxidation were used during the cathode phase for oxygen reduction. In parallel, Buisman's group developed a solar microbial cathode, which showed similar reversibility.[90] The electrode was first inoculated with a nitrifying sludge, which was thereafter illuminated and further inoculated with phototrophic microorganisms. During the dark period, in which the microbial electrode produced an anodic current, the pH value of the bulk solution dropped due to the accumulation of the protons produced. During illumination, the phototrophic biofilm, which contained algae, cyanobacteria and other bacteria, consumed CO_2 and produced oxygen, which was locally reduced by the microbial cathode. In this period, the pH value increased as a result of the consumption of protons by oxygen reduction. The pH value of the reversible electrode thus oscillated between 6.7 and 7.2 without pH control for a period of 22 days. Once again, the protons required for oxygen reduction were provided in situ during the anode period. The concept is interesting, but further research is needed to explore the application of such bidirectional microbial electrodes.[89]

6.2.2. Oxygen transfer

Oxygen transfer can clearly be promoted by stirring the catholyte or increasing the catholyte flow rate in continuous systems.[80] Using an open air cathode[35] is a relevant and perhaps the most effective way to enhance oxygen availability on the cathode surface. In this case, the gas diffusion layer becomes a core component of the cell to promote a uniform access of oxygen to the catalyst[91] and with complex other roles such as removing by-produced vapour and preventing water crossover. Using pure oxygen resulted in high currents.[54] It may be guessed that a too-high oxygen concentration may favour the formation of reactive oxygen species that are detrimental to living cells, but it appears that aerobic biofilms possess an arsenal of enzymes to protect them against oxidative stress. Finally, the solar microbial reversible cathode developed by Buisman's group[90] (see paragraph above) is a very elegant way to solve the problem of slow oxygen transfer by producing oxygen directly on the cathode surface. Current density and Coulombic efficiency related to oxygen consumption were quite low, but the stability of the system (reversible anode and cathode functioned for 63 days) showed that the concept deserves further investigation.

6.2.3. Sensitivity to reactive oxygen species

On this issue, the numerous studies carried out in the field of MIC are reassuring. It seems that aerobic biofilms possess a large diversity of proteins (catalase, superoxide dismutase, peroxidases…) that catalyse the elimination of reactive oxygen

species (reactions 9–11). The electroactive biofilms may thus be naturally protected from producing too high concentrations of reactive oxygen species.

6.2.4. Fouling due to alkalinisation of the interface and biofouling

This problem has certainly not been sufficiently considered as yet. Chemical fouling[34,92] and also biofouling due to heterotrophic bacteria encouraged by rich organic feeding[89] have already been observed. Nevertheless, (bio)fouling still seems underestimated in laboratory experiments that use synthetic media. It may become a severe constraint when trying to transfer microbial cathodes to actual environments.

Finally, it can be concluded that investigation of microbial cathodes for ORR catalysis has so far been modest with respect to their great interest. Combined approaches, associating electrochemistry, chemistry, microbiology, biology, ecology, material sciences and engineering, need to be extensively pursued to explore the numerous seminal technologies that have been described and, hopefully, to open up new knowledge-based paths.

Acknowledgements

The authors gratefully acknowledge the late Dr. Vittoria Scotto and Dr. Alfonso Mollica (CNR Genoa, Italy) for very helpful discussions. The article has benefited greatly from their extensive experience and their great scientific enthusiasm. A part of the article has been written in the framework of the "Agri-Elec (ANR-008-BioE-001)" project, supported by the French ANR.

Keywords: electrochemistry · fuel cells · microbes · oxygen reduction · thin films

[1] a) M. C. Potter, *Proc. R. Soc. London Ser. B* **1911**, *84*, 260 – 276; b) J. B. Davis, H. F. Yarbrough, Jr., *Science* **1962**, *137*, 615 – 616.
[2] B. H. Kim, T. Ikeda, H. S. Park, H. J. Kim, M. S. Hyun, K. Kano, K. Takagi, H. Tatsumi, *Biotechnol. Tech.* **1999**, *13*, 475 – 478.
[3] a) D. R. Bond, D. E. Holmes, L. M. Tender, D. R. Lovley, *Science* **2002**, *295*, 483 – 485; b) L. M. Tender, C. E. Reimers, H. A. Stecher, III, D. E. Holmes, D. R. Bond, D. A. Lowy, K. Pilobello, S. J. Fertig, D. R. Lovley, *Nat. Biotechnol.* **2002**, *20*, 821 – 825.
[4] a) K. Rabaey, J. Rodriguez, L. L. Blackall, J. Keller, P. Gross, D. Batstone, W. Verstraete, K. H. Nealson, *ISME J.* **2007**, *1*, 9 – 18; b) B. E. Logan, *Nat. Rev. Microbiol.* **2009**, *7*, 375 – 381; c) A. E. Franks, K. P. Nevin, *Energies* **2010**, *3*, 899 – 919; d) D. R. Lovley, K. P. Nevin, *Curr. Opin. Biotechnol.* **2011**, *22*, 441 – 448.
[5] F. Zhao, F. Harnisch, U. Schröder, F. Scholz, P. Bogdanoff, I. Herrmann, *Environ. Sci. Technol.* **2006**, *40*, 5193 – 5199.
[6] K. Kinoshita, *Electrochemical Oxygen Technology*, Wiley Interscience, **1992**.
[7] M. J. Cooney, V. Svoboda, C. Lau, G. Martin, S. D. Minteer, *Energy Environ. Sci.* **2008**, *1*, 320 – 337.
[8] O. Schaetzle, F. Barrière, U. Schröder, *Energy Environ. Sci.* **2009**, *2*, 96 – 99.
[9] F. Harnisch, U. Schröder, *Chem. Soc. Rev.* **2010**, *39*, 4433 – 4448.
[10] Z. He, L. T. Angenent, *Electroanalysis* **2006**, *18*, 2009 – 2015.
[11] I. B. Beech, J. Sunner, *Curr. Opin. Biotechnol.* **2004**, *15*, 181 – 186.
[12] J. Landoulsi, K. El Kirat, C. Richard, D. Féron, S. Pulvin, *Environ. Sci. Technol.* **2008**, *42*, 2233 – 2242.
[13] B. J. Little, J. S. Lee, R. I. Ray, *Electrochim. Acta* **2008**, *54*, 2 – 7.
[14] V. Sharma, P. P. Kundu, *Enzyme Microb. Technol.* **2010**, *47*, 179 – 188.
[15] L. Huang, J. M. Regan, X. Quan, *Bioresour. Technol.* **2011**, *102*, 316 – 323.
[16] M. Rosenbaum, F. Aulenta, M. Villano, L. T. Angenent, *Bioresour. Technol.* **2011**, *102*, 324 – 333.
[17] A. P. Borole, G. Reguera, B. Ringeisen, Z.-W. Wang, Y. Feng, B. H. Kim, *Energy Environ. Sci.* **2011**, DOI:10.1039/c1ee02511b.
[18] F. L. LaQue, *J. Am. Naval Eng.* **1941**, *53*, 29 – 64.
[19] N. S. Nikitina, I. B. Ulanovskii, *Biol. Sta. Akad. Nauk. SSSR* **1957**, *3*, 190 – 199 .
[20] K. P. Fischer, E. Rislund, O. Steensland, U. Steinsmo, B. Wallen, *European Federation of Corrosion Publications* **2001**, *33*, The Institute of Materials, London, pp. 3 – 11.
[21] a) A. E. Zaragoza-Ayala, N. Acuna, W. Solis, J. Aldana, D. Festy, *Corrosion-NACE* **1996**, paper 504; b) N. Acuna, G. Hernandez-Duque, *Corrosion-NACE* **1998**, paper 698.
[22] G. Alabiso, V. Scotto, A. Mollica, U. Montini, G. Marcenaro, R. Dellepiane *European Federation of Corrosion Publications* **1996**, *19*, The Institute of Materials, London, pp. 95 – 105.
[23] W. H. Dickinson, F. Caccavo, Z. Lewandowski, *Corros. Sci.* **1996**, *38*, 1407 – 1422.
[24] Z. G. Chen, P. Gümpel, M. Kässer, R. Kreikenbohm R, *European Federation of Corrosion Publications* **1996**, *22*, The Institute of Materials, London, pp. 125 – 134.
[25] Y. Ishihara, S. Tsujikawa, *Corros. Eng.* **1999**, *48*, 703 – 712.
[26] A. Iversen, *Br. Corros. J.* **2001**, *36*, 277 – 291.
[27] A. Mollica, *Int. Biodeterior. Biodegrad.* **1992**, *29*, 213 – 229.
[28] A. Mollica, A. Trevis A, *Proceedings of the 4th Int. Congress on Marine Corrosion and Fouling* **1976**, Antibes, France, pp. 351 – 365.
[29] a) F. Mansfeld, R. Tsai, H. Shih, B. J. Little, R. Ray, P. Wagner, *Corros. Sci.* **1992**, *33*, 445 – 456; b) G. Salvago, L. Magagnin, *Corrosion* **2001**, *57*, 680 – 692.
[30] a) S. C. Dexter, G. Y. Gao, *Corrosion* **1988**, *44*, 717 – 723; b) F. L. Roe, Z. Lewandowski, T. Funk, *Corrosion* **1996**, *52*, 744 – 754; c) S. C. Dexter, J. P. La Fontaine, *Corrosion* **1998**, *54*, 851 – 861.
[31] A. Mollica, E. Traverso, D. Thierry, *European Federation of Corrosion Publications* **1997**, *22*, The Institute of Materials, London, pp. 51 – 63.
[32] a) K. P. Fischer, E. Rislund, O. Steensland, U. Steinsmo, B. Wallen, *European Federation of Corrosion Publications* **2001**, *33*, The Institute of Materials, London, pp. 3 – 11; b) V. Scotto, A. Mollica, J. P. Audouard, C. Compère, D. Féron, D. Festy, T. Rogne, U. Steinsmo, K. Taxen, D. Thierry, *Proceedings of Eurocorr'96*, *Nice*, *France* **1996**, *14*, paper OR2.
[33] O. Hasvold, H. Henriksen, E. Melvaer, G. Citi, B. O. Johansen, T. Kjonigsen, R. Galetti, *J. Power Sources* **1997**, *65*, 253 – 261.
[34] a) A. Bergel, D. Féron, A. Mollica, *Electrochem. Commun.* **2005**, *7*, 900 – 904; b) A. Bergel, D. Féron, *CNRS-CEA French Patent*, **2002**, FR 02 10009; PCT/IB03/03637.
[35] P. Clauwaert, D. Van der Ha, N. Boon, K. Verbeken, M. Verhaege, K. Rabaey, W. Verstraete, *Environ. Sci. Technol.* **2007**, *41*, 7564 – 7569.
[36] G.-W. Chen, S.-J. Choi, T.-H. Lee, G. Y. Lee, J.-H. Cha, C.-W. Kim, *Appl. Microbiol. Biotechnol.* **2008**, *79*, 379 – 388.
[37] A. Aldrovandi, E. Marsili, L. Stante, P. Paganin, S. Tabacchioni, A. Giordano, *Bioresour. Technol.* **2009**, *100*, 3252 – 3260.
[38] P. Liang, M. Fan, X. Cao, X. Huang, *J. Chem. Technol. Biotechnol.* **2009**, *84*, 794 – 799.
[39] S.-J. You, N.-Q. Ren, Q.-L. Zhao, J.-Y. Wang, F.-L. Yang, *Fuel Cells* **2009**, *9*, 588 – 596.
[40] J. Cha, S. Choi, H. Yu, H. Kim, C. Kim, *Bioelectrochemistry* **2010**, *78*, 72 – 79.
[41] a) G.-W. Chen, J.-H. Cha, S.-J. Choi, T.-H. Lee, G. Y. Lee, C.-W. Kim, *Korean J. Chem. Eng.* **2010**, *27*, 828 – 835; b) G.-W. Chen, S.-J. Choi, J.-H. Cha, T.-H. Lee, C.-W. Kim, *Korean J. Chem. Eng.* **2010**, *27*, 1513 – 1520.
[42] K. Y. Cheng, G. Ho, R. Cord-Ruwisch, *Environ. Sci. Technol.* **2010**, *44*, 518 – 525.
[43] A. Ter Heijne, D. Strik, H. Hamelers, C. Buisman, *Environ. Sci. Technol.* **2010**, *44*, 7151 – 7156.
[44] J. Wei, P. Liang, X. Cao, X. Huang, *Bioresour. Technol.* **2011**, *102*, 10431 – 10435.
[45] a) C. Dumas, A. Mollica, D. Féron, R. Basséguy, L. Etcheverry, A. Bergel, *Electrochim. Acta* **2007**, *53*, 468 – 473; b) M. Faimali, E. Chelossi, G. Pava-

nello, A. Benedetti, I. Vandecandelaere, P. De Vos, P. Vandamme, A. Mollica, *Bioelectrochemistry* **2010**, *78*, 30 – 38.
[46] a) M. Faimali, E. Chelossi, F. Garaventa, C. Corra, G. Greco, A. Mollica, *Electrochim. Acta* **2008**, *54*, 148 – 153; b) C. Dumas, A. Mollica, D. Féron, R. Basséguy, L. Etcheverry, A. Bergel, *Bioresour. Technol.* **2008**, *99*, 8887 – 8894.
[47] K. Rabaey, S. T. Read, P. Clauwaert, S. Freguia, P. L. Bond, L. L. Blackall, J. Keller, *ISME J.* **2008**, *2*, 519 – 527.
[48] L. De Schamphelaire, P. Boeckx, W. Verstraete, *Appl. Microbiol. Biotechnol.* **2010**, *87*, 1675 – 1687.
[49] B. Erable, I. Vandecandelaere, M. Faimali, M.-D. Délia, L. Etcheverry, P. Vandamme, A. Bergel, *Bioelectrochemistry* **2010**, *78*, 51 – 56.
[50] R. Renslow, C. Donovan, M. Shim, J. Babauta, S. Nannapaneni, J. Schenk, H. Beyenal, *Phys. Chem. Chem. Phys.* **2011**, *13*, 21573 – 21584.
[51] T.-S. Song, H.-L. Jiang, *Bioresour. Technol.* **2011**, *102*, 10465 – 10470.
[52] Z. Chen, Y.-C. Huang, J.-H. Liang, F. Zhao, Y.-G. Zhu, *Bioresour. Technol.* **2012**, *108*, 55 – 59.
[53] G.-D. Zhang, Q.-L. Zhao, Y. Jiao, J. Zhang, N. Ren, B. H. Kim, *J. Power Sources* **2011**, *196*, 6036 – 6041.
[54] S. Carbajosa, M. Malki, R. Caillard, M. F. Lopez, F. J. Palomares, J. A. Martin-Gago, N. Rodriguez, R. Amils, V. M. Fernandez, A. L. De Lacey, *Biosens. Bioelectron.* **2010**, *26*, 877 – 880.
[55] a) A. Cournet, M. Bergé, C. Roques, A. Bergel, M.-L. Délia, *Electrochim. Acta* **2010**, *55*, 4902 – 4908; b) A. Cournet, M.-L. Délia, A. Bergel, C. Roques, M. Bergé, *Electrochem. Commun.* **2010**, *12*, 505 – 508.
[56] S. Freguia, S. Tsujimura, K. Kano, *Electrochim. Acta* **2010**, *55*, 813 – 818.
[57] Y. Mao, L. Zhang, D. Li, H. Shi, Y. Liu, L. Cai, *Electrochim. Acta* **2010**, *55*, 7804 – 7808.
[58] A. Rhoads, H. Beyenal, Z. Lewandowski, *Environ. Sci. Technol.* **2005**, *39*, 4666 – 4671.
[59] a) V. Scotto, R. Di Cintio, G. Marcenaro, *Corros. Sci.* **1985**, *25*, 185 – 194; b) V. Scotto, M. Beggiato, G. Marcenaro, R. Dellepiane, *European Federation of Corrosion Publications* **1993**, *10*, The Institute of Materials, London, pp. 21 – 33; c) V. Scotto, M. E. Lai, *European Federation of Corrosion Publications* **1997**, *22*, The Institute of Materials, London, pp. 41 – 49; d) V. Scotto, M. E. Lai, *Corros. Sci.* **1998**, *40*, 1007 – 1018.
[60] a) M. E. Lai, A. Bergel, *J. Electroanal. Chem.* **2000**, *494*, 30 – 40; b) R. Huang, N. Hu, *Bioelectrochemistry* **2001**, *54*, 75 – 81.
[61] a) R. Jiang, S. Dong, *Electrochim. Acta* **1990**, *35*, 1227 – 1232; b) F. Arifuku, K. Mori, T. Muratami, H. Kurihara, *Bull. Chem. Soc. Jpn.* **1992**, *65*, 1491 – 1495.
[62] S. Parot, I. Vandecandelaere, A. Cournet, M.-L. Délia, P. Vadamme, M. Bergé, C. Roques, A. Bergel, *Bioresour. Technol.* **2011**, *102*, 304 – 311.
[63] a) R. Basséguy, J. Idrac, C. Jacques, A. Bergel, M.-L. Delia, L. Etcheverry, *European Federation of Corrosion Publications* **2007**, *45*, The Institute of Materials, p. 52; b) H. Iken, L. Etcheverry, A. Bergel, R. Basséguy, *Electrochim. Acta* **2008**, *54*, 60 – 65.
[64] G. Manzini, A. Cesaro, F. Delben, S. Paoletti, E. Reisenhofer, *Bioelectrochem. Bioenerg.* **1984**, *12*, 443 – 454.
[65] R. Johnson, E. Bardal, *Corrosion* **1985**, *41*, 296 – 309.
[66] F. Van den Brink, E. Barendrecht, W. Visscher, *Rec. J. R. Neth. Chem. Soc.* **1980**, *99*, 253 – 262.
[67] P. Chandrasekaran, S. C. Dexter, 12th Int. Corr. Congress, **1993**, *5B*, Houston, TX, 3696 – 3707.
[68] M. Denis, S. Arnaud, F. Malatesta, *FEBS Lett.* **1989**, *247*, 475 – 483.
[69] a) W. H. Dickinson, Z. Lewandoski, R. D. Geer, *Corrosion* **1996**, *52*, 910 – 920; b) K. Xu, S. C. Dexter, G. W. Luther, *Corrosion* **1998**, *54*, 814 – 823; c) N. Washizu, Y. Katada, T. Kodama, *Corros. Sci.* **2004**, *46*, 1291 – 1300.
[70] a) H. Amaya, H. Miyuki, *Corros. Eng.* **1995**, *44*, 123 – 132; b) H. Amaya, H. Miyuki, *Corros. Eng.* **1997**, *46*, 567 – 581.
[71] a) D. Féron, I. Dupont, G. Novel, *European Federation of Corrosion Publications* **1997**, *22*, The Institute of Materials, London, pp. 103 – 122; b) I. Dupont, D. Féron, G. Novel, *Int. Biodeterior. Biodegrad.* **1998**, *41*, 13 – 18; c) V. L'Hostis, C. Dagbert, D. Féron, *Electrochim. Acta* **2003**, *48*, 1451 – 1458; d) W. Wang, X. Zhang, J. Wang, *Electrochim. Acta* **2009**, *54*, 5598 – 5604.
[72] a) W. H. Dickinson, Z. Lewandowski, *Biofouling* **1996**, *10*, 79 – 93; b) B. H. Olesen, R. Avci, Z. Lewandowski, *Corros. Sci.* **2000**, *42*, 211 – 227.
[73] X. Shi, R. Avci, Z. Lewandowski, *Corros. Sci.* **2002**, *44*, 1027 – 1045.
[74] K. R. Braughton, R. L. Lafond, Z. Lewandowski, *Biofouling* **2001**, *17*, 241 – 251.
[75] a) M. da Cunha Belo, B. Rondot, C. Compère, M. F. Montemor, A. M. P. Simoes, M. G. S. Ferreira, *Corros. Sci.* **1998**, *40*, 481 – 494; b) N. Le Bozec, C. Compère, M. L'Her, A. Laouenan, D. Costa, P. Marcus, *Corros. Sci.* **2001**, *43*, 765 – 786; c) C. Poleunis, C. Compère, P. Bertrand, *J. Microbiol. Methods* **2002**, *48*, 195 – 205.
[76] D. E. Caldwell, D. R. Korber, J. R. Lawrence, *Adv. Microb. Ecol.* **1992**, *12*, 1 – 67.
[77] a) B. Little, R. Ray, P. Wagner, Z. Lewandowski, W. C. Lee, W. G. Characklis, F. Mansfeld, *Biofouling* **1991**, *3*, 45 – 59; b) M. Eashwar, S. Maruthamuthu, *Biofouling* **1995**, *8*, 203 – 213.
[78] E. Bardal, J. M. Drugli, P. O. Gartland, *Corr. Sci.* **1993**, *35*, 257 – 267.
[79] D. Féron in *Microbially influenced corrosion and biodeterioration, International Congress on Microbially Influenced Corrosion (Knoxville)* **1990**, 5, pp. 73 – 84.
[80] A. Ter Heijne, O. Schaetzle, S. Gimenez, F. Fabregat-Santiago, J. Bisquert, D. P. B. T. B. Strik, F. Barriere, C. J. N. Buisman, H. V. M. Hamelers, *Energy Environ. Sci.* **2011**, *4*, 5035 – 5043.
[81] T. P. Curtis, W. T. Sloan, J. W. Scannell, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 10494 – 10499.
[82] E. Lyautey, A. Cournet, S. Morin, S. Boulêtreau, L. Etcheverry, J.-Y. Charcosset, F. Delmas, A. Bergel, F. Garabetian, *Appl. Environ. Microbiol.* **2011**, *77*, 5394 – 5401.
[83] R. I. Amann, W. Ludwig, K. H. Schleifer, *Microbiol. Rev.* **1995**, *59*, 143 – 169.
[84] I. Vandecandelaere, O. Nercessian, M. Faimali, E. Segaert, A. Mollica, W. Achouak, P. De Vos, P. Vandamme, *Bioelectrochemistry* **2010**, *78*, 62 – 66.
[85] J. Wei, P. Liang, X. Huang, *Bioresour. Technol.* **2011**, *102*, 9335 – 9344.
[86] S. Parot, O. Nercessian, M.-L. Délia, Achouak, A. Bergel, *J. Appl. Microbiol.* **2009**, *106*, 1350 – 1359.
[87] X.-W. Liu, X.-F. Sun, Y.-X. Huang, G.-P. Sheng, S.-G. Wang, H.-Q. Yu, *Energy Environ. Sci.* **2011**, *4*, 1422 – 1427.
[88] B. Erable, L. Etcheverry, A. Bergel, *Electrochem. Commun.* **2009**, *11*, 619 – 622.
[89] S. Freguia, K. Rabaey, Z. Yuan, J. Keller, *Water Res.* **2008**, *42*, 1387 – 1396.
[90] D. P. B. T. B. Strik, H. V. M. Hamelers, C. J. N. Buisman, *Environ. Sci. Technol.* **2010**, *44*, 532 – 537.
[91] a) G. Gupta, C. Lau, B. Branch, V. Rajendran, D. Ivnitski, P. Atanassov, *Electrochim. Acta* **2011**, *56*, 10767 – 10771; b) T. Miyake, K. Haneda, N. Nagai, Y. Yatagawa, H. Onami, S. Yoshino, T. Abe, M. Nishizawa, *Energy Environ. Sci.* **2011**, *4*, 5008 – 5012.
[92] K. Chung, I. Fujiki, S. Okabe, *Bioresour. Technol.* **2011**, *102*, 355 – 360.